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New chemical and chemo-enzymatic routes for the synthesis of (*RS*)- and (*S*)-enciprazine

Linga Banoth, Thete Karuna Narayan, Uttam C. Banerjee *

Biocatalysis Laboratory, Department of Pharmaceutical Technology, National Institute of Pharmaceutical Education and Research, Sector-67, S.A.S. Nagar 160062, Punjab, India

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ABSTRACT

The chemo-enzymatic synthesis of racemic and enantiopure (*RS*)- and (*S*)-enciprazine **1**, a non-benzodiazepine anxiolytic drug, is described herein. The synthesis started from 1-(2-methoxyphenyl) piperazine **3**, which was treated with 2-(chloromethyl) oxirane (*RS*)-**4** using lithium bromide to afford a racemic alcohol, 1-chloro-3-(4-(2-methoxyphenyl) piperazin-1-yl) propan-2-ol (*RS*)-**6** in 85% yield. Intermediate (*S*)-**6** was synthesized from racemic alcohol (*RS*)-**6** using *Candida rugosa* lipase (CRL) with vinyl acetate as the acyl donor. Various reaction parameters such as temperature, time, substrate, enzyme concentration, and the effect of the reaction medium on the conversion and enantiomeric excess for the transesterification of (*RS*)-**6** by CRL were optimized. It was observed that 10 mM of (*RS*)-**6**, 50 mg/mL of CRL in 4.0 mL of toluene with vinyl acetate (5.4 mmol) as acyl donor at 30 °C gave good conversion ($C = 49.4\%$) and enantiomeric excess ($ee_P = 98.4\%$ and $ee_S = 96\%$) after 9 h of reaction. Compound (*S*)-**6** is a key intermediate for the synthesis of enantiopure (*S*)-**1**. The (*RS*)- and (*S*)-enciprazine drug **1** was synthesized by treating (*RS*)- and (*S*)-**6** with 3,4,5-trimethoxyphenol **5** using MeCN as a solvent and K_2CO_3 as a base.

1. Introduction

Optically active aryloxypropanols have been widely employed as starting materials for the synthesis of β-blockers, which are used in the management of sympathetic nervous system associated cardiovascular[1] disorders such as hypertension,[2] angina pectoris, cardiac arrhythmias, and other disorders[3] such as depression, loss of appetite, asthma, migraine, glaucoma, and so on. All β-blockers have at least one stereogenic center in their structure and so, have two enantiomers.[4] However, some β-adrenergic blocking agents cross the blood–brain barrier because of their lipophilicity and act on central nervous system functions.[5] Propranolol has long been used as a drug for the treatment of prophylaxis of migraine headaches, anxiety syndromes, alcohol withdrawal, schizophrenia, and tremors.[6] The newly developed aryloxypropanol derivatives with various heterocycles in the hydrophilic moiety of the molecule have shown anticonvulsive activity. Enciprazine **1** (Scheme 1) is a non-benzodiazepine anxiolytic drug.[7] It has very little sedative/hypnotic side effects and its interaction with alcohol is far less than that of the benzodiazepines.[8] Enciprazine exhibits a variety of biological activities, such as cardiovascular, hypotensive, and local anesthetic properties.[9] The different approaches for the synthesis of **1** are summarized in Scheme 1.[10]

In general, the synthesis of the 1,2-aminoalcohol class of β-adrenergic blocking agents involves the opening of an epoxide ring with amines.[11] The synthesis of enciprazine **1** involves the epoxide ring opening reaction of *rac*-2-[(3,4,5,-trimethoxyphenoxy)methyl] oxirane **2** with 1-(2-methoxyphenyl)piperazine **3**. The methodology for the synthesis of enantiopure/enantio-enriched **1** involves the epoxide ring opening reaction of the glycidic ether (*S*)-**2**, prepared by the hydrolytic kinetic resolution of (*RS*)-**2** using an enantioenriched (*R*,*R*)-salen–Cobalt(III) complex. The glycidic ether (*RS*)-**2** was synthesized by treatment of epichlorohydrin **4** with 3,4,5-trimethoxy phenol **5** in the presence of a base. The phenol **5** itself was synthesized by a two step procedure starting from 3,4,5-trimethoxybenzaldehyde.[10] This reported synthesis has drawbacks, such as it being a multistep procedure for the preparation of **2**, which requires an expensive and toxic (*R*,*R*)-salen–Co(OAc) complex which is carcinogenic and flammable.[10] New chemical and chemo-enzymatic routes for the synthesis of (*RS*)- and (*S*)-enciprazine (Scheme 1) are discussed herein.

2. Results and discussion

The increasing influence of green chemistry tools on the synthesis of new chemical entities and for the improvement of existing chemical synthesis[12–13] is now widely recognized. Integrating biocatalysis in chemical syntheses is an elegant approach in expanding the organic synthesis toolbox.[14] *N*-Heterocyclic amines are very much in demand for the synthesis of various agro- and fine

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* Corresponding author. Tel.: +91 172 2214682 85x2142; fax: +91 172 2214692.
E-mail address: ucbanerjee@niper.ac.in (U.C. Banerjee).

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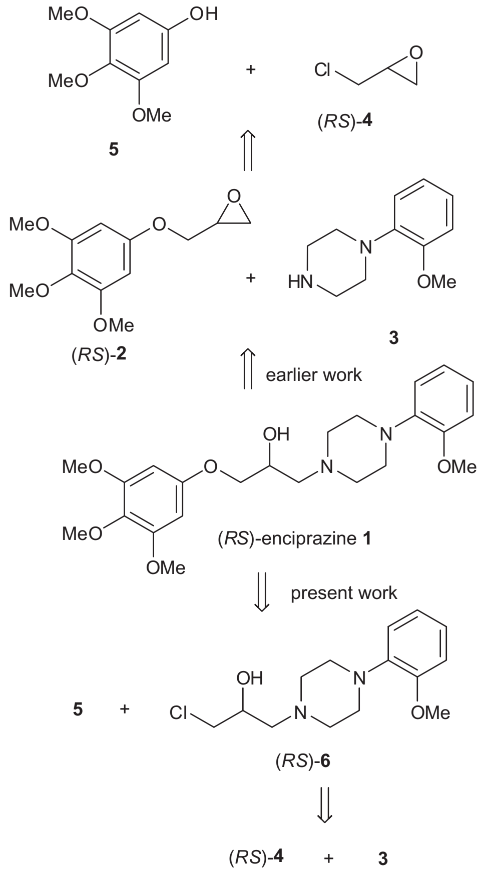


Scheme 1. Various synthetic strategies for **1**.

chemicals and pharmaceuticals.[15] They also have remarkable properties in organocatalytic reactions. It has been reported in the literature[15] that highly valuable, optically active cyclic amines, such as piperidines, pyrrolidines, azetidines, aziridines, and biologically active compounds such as antibiotics, antihypertensive drugs, and alkaloids can be synthesized by lipase-catalyzed stereoselective acylations. The enzymatic kinetic resolution of a racemic secondary alcohol provides[16] a new chemo-enzymatic route for the synthesis of (*S*)-enciprazine (Scheme 2).

2.1. Synthesis of (*RS*)-1-chloro-3-(4-(2-methoxyphenyl)piperazin-1-yl) propan-2-ol 6

As shown in the retrosynthetic analysis (Scheme 2), the synthesis started from the commercially available 1-(2-methoxyphenyl) piperazine **3**, which was treated with commercially available 2-(chloromethyl) oxirane (*RS*)-**4** using lithium bromide to afford racemic alcohol (*RS*)-**6** in 85% yield (Scheme 3) as the substrate required for enzymatic kinetic resolution.

For the kinetic resolution of (*RS*)-**6**, authentic samples of (*R*)- and (*S*)-**6** and the corresponding O-acylated derivatives (*RS*), (*R*)-, and (*S*)-**7** were required.

2.2. Synthesis of authentic samples of (*R*)- and (*S*)-1-chloro-3-(4-(2-methoxyphenyl)piperazin-1-yl)propan-2-ol 6 and (*RS*)-, (*R*)- and (*S*)-1-chloro-3-(4-(2-methoxyphenyl) piperazin-1-yl) propan-2-yl acetate 7

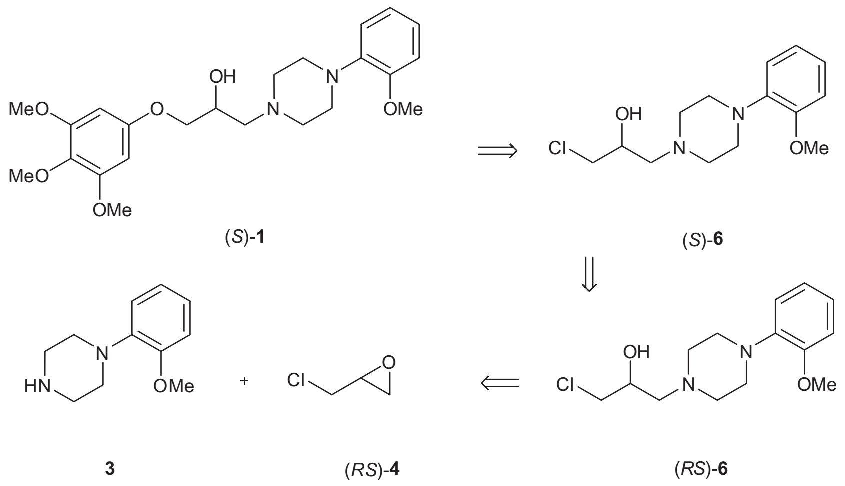
The authentic samples of (*R*)- and (*S*)-**6** were synthesized by the reaction of **3** with (*R*)- and (*S*)-**4**, respectively. The enantiomeric purity was determined by chiral HPLC and by the specific rotation value. The ring opening of (*S*)-**4** by treatment with **3** following the same procedure as for (*RS*)-**6** afforded (*S*)-**6** in 83% yield and with 92% ee. Similarly, (*R*)-**6** was obtained from (*R*)-**4** in 84% yield and with 91% ee. The treatment of (*RS*)-**6** with acetic anhydride at 4 °C in the presence of pyridine afforded (*RS*)-**7** in 90% yield. Acetylation of (*R*)- and (*S*)-**6** following a similar procedure resulted in the formation of (*R*)- and (*S*)-**7** in 92 and 91% yields with 87% and 89% ee, respectively.

2.3. Lipase-catalyzed kinetic resolution of (*RS*)-6

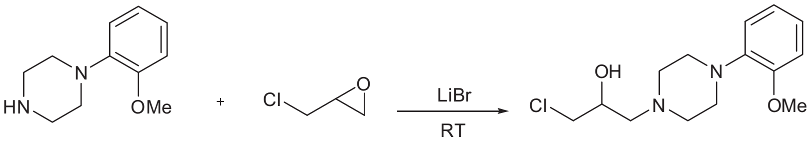
The best operative enzymatic kinetic resolution procedure using various lipases was followed with substrates (*RS*)-**6** and the authentic samples of (*R*)- and (*S*)-**6** and (*RS*)-, (*R*)-, and (*S*)-**7**.

2.3.1. Screening of lipases

Commercially available lipases, such as *Pseudomonas cepacia* immobilized on sol-gel-Ak (PS-C), immobilized lipozyme *Mucor miehei*, *Candida antarctica* immobilized on acrylic resin (CAL-B), lipase AY 'Amano'30, lipase A from *Candida antarctica* (CAL-A) and lipase from *Candida rugosa* (L8525), *Candida cylindracea*, *Aspergillus niger*, and porcine pancreas lipase were screened for the transesterification of (*RS*)-**6** with vinyl acetate in toluene (Scheme 4).

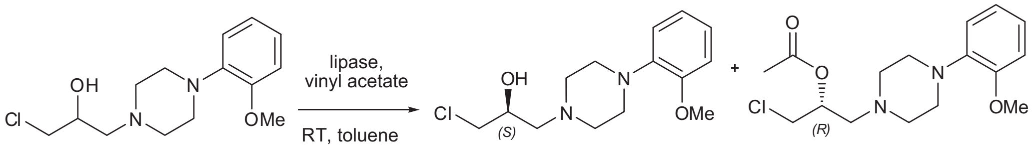


Scheme 2. New chemo-enzymatic route for (*RS*)- and (*S*)-enciprazine.



3 (*RS*)-**4** (*RS*)-**6**

Scheme 3. Synthesis of (*RS*)-**6**.



(*RS*)-**6** (*S*)-**6** (*R*)-**7**

Scheme 4. Enzymatic kinetic resolution of (*RS*)-**6**.

Lipases CRL, CAL, and PCL exhibited the best activity for the conversion of (*RS*)-**6** to (*R*)-**7** and (*S*)-**6**. However, CRL was found to be better than CAL and PCL in terms of conversion and enantiomeric excess (Table 1).

Table 1
Lipase-catalyzed transesterification of (*RS*)-**6** with vinyl acetate[a]

Lipase[b]	Time (h)	C[c] (%)	ee_s[d] (%)	ee_p[e] (%)	*E*[f]
CAL	24	18.7	21.2	91.9	29.4
PCL	24	26.4	32.5	90.6	27.9
CRL	24	29.7	37.8	89.5	26.2

[a] Conditions: (*RS*)-**6** (20 mM) in 4 mL of toluene was treated with vinyl acetate (5.4 mmol) at 25 °C in the presence of the enzyme (25 mg/mL of CAL, PCL, and CRL).

[b] PCL (immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia*), CAL (lipase acrylic resin from *Candida antarctica*), and CRL (lipase from *Candida rugosa*).

[c] Conversions were calculated from the enantiomeric excess (ee) of (*S*)-**6** (substrate S) and (*R*)-**7** (product P) using the formula: conversion (C) = $ee_s/(ee_s + ee_p)$.

[d] Enantiomeric excess of (*S*)-**6** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at 261 nm.

[e] Enantiomeric excess of (*R*)-**7** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at 261 nm.

[f] *E* values were calculated using the formula: $E = [\ln(1 - C(1 + ee_P)]/[\ln(1 - C(1 - ee_P)]$.[17]

Table 2
The effect of the organic solvent on the enantioselectivity in the resolution of (*RS*)-**6** with lipase CRL[a]

Solvent	C[b] (%)	ee_s[c] (%)	ee_p[d] (%)	*E*[e]
Acetone	4.0	3.4	80.2	9.4
Diethyl ether	29.5	35.1	83.7	15.8
Dichloromethane	17.1	19.7	95.5	52.5
Diisopropyl ether	26.1	31.6	89.5	24.5
Benzene	32.5	43.9	91.1	32.9
Chloroform	39.0	59.4	92.8	48.64
Toluene	41.2	62.7	89.7	34.9
Carbon tetrachloride	21.5	25.4	92.7	33.6
Cyclo hexane	27.8	35.1	90.9	29.6
Hexane	30.3	38.9	89.7	26.9
Heptane	30.1	38.5	89.5	26.4
iso-Octane	29.4	37.6	90.5	28.0

[a] Conditions: (*RS*)-**6** (20 mM) in toluene (4 mL) was treated with vinyl acetate (5.4 mmol) at 25 °C in the presence of lipase CRL (25 mg/mL).

[b] Conversions were calculated from the enantiomeric excess (ee) of (*S*)-**6** (substrate S) and (*R*)-**7** (product P) using the formula: conversion (C) = $ee_s/(ee_s + ee_p)$.

[c] Enantiomeric excess of (*S*)-**6** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at 261 nm.

[d] Enantiomeric excess of (*R*)-**7** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at 261 nm.

[e] *E* values were calculated using the formula: $E = [\ln(1 - C(1 + ee_P)]/[\ln(1 - C(1 - ee_P)]$.[17]

2.3.2. Selection of the organic solvent

The effect of various solvents (varying log*P* values such as toluene, *iso*-octane, chloroform, dichloromethane, etc) on the enantioselectivity of enzymatic reaction,[18] was investigated by transesterification of (*RS*)-**6** with vinyl acetate in the presence of CRL (Table 2). The maximum enantiomeric excess of substrate was observed in toluene.

2.3.3. Effect of reaction time

A time dependent CRL catalyzed transesterification reaction of (*RS*)-**6** with vinyl acetate was performed in toluene. Samples were collected periodically from the reaction mixture and centrifuged at 10,000×g for 5 min to remove the enzyme. The conversion and enantiomeric excess were determined using chiral HPLC. It can be seen from Table 1 that the conversion increased with time and the maximum conversion ($C = 45.9\%$) and ee_s (67.8%) were achieved at 9 h, after which no significant change in the rate of conversion or ee_s was observed. On the other hand, the ee_p of the product decreased with time ($ee_p = 80.6\%$ at 6 h to 79.6% at 9 h) (Fig. 1). Thus, 9 h was chosen as the optimal reaction time.

2.3.4. Effect of acyl donors

The effect of acyl donors on the conversion rate and ee of the CRL-catalyzed kinetic resolution of (*RS*)-**6** was studied in toluene (Table 3). The best results were obtained using vinyl acetate compared to other acyl donors. The superiority of vinyl acetate as the acyl donor for the enzymatic reaction is because of the in situ tautomerization ability of vinyl alcohol to acetaldehyde, and so it does not act as a competitive substrate and shifts the equilibrium toward the product.[19]

2.3.5. Effect of temperature

The influence of temperature on the activity and enantioselectivity of the CRL-catalyzed kinetic resolution of (*RS*)-**6** using vinyl acetate as the acyl donor in toluene was determined (Fig. 2). The

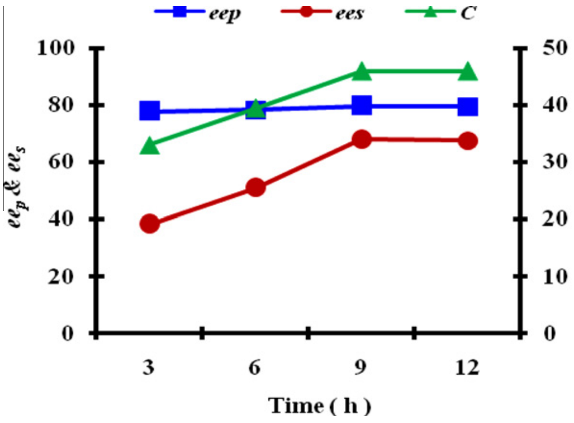


Figure 1. Course of CRL catalyzed transesterification of (*RS*)-**6** in toluene.

Table 3
The effect of acyl donors on the CRL catalyzed transesterification of (*RS*)-**6** in toluene[a]

Acyl donors[a]	C[b] (%)	ee_s[c] (%)	ee_p[d] (%)
Phenylethylacetate	9	8	100
Ethyl acetate	3.3	3.4	100
Methylchloroacetate	5.4	5.8	100
Acetic anhydride	0.6	0.6	100
Vinyl acetate	34.2	41.7	80.2

[a] Conditions: (*RS*)-**6** (20 mM) in toluene (4 mL) was treated with the acyl donor (5.4 mmol) at 25 °C in the presence of lipase CRL (25 mg/mL).

[b] Conversions were calculated from the enantiomeric excess (ee) of (*S*)-**6** (substrate S) and (*R*)-**7** (product P) using the formula: conversion $(C) = ee_s/(ee_s + ee_p)$.

[c] Enantiomeric excess of (*S*)-**6** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at 261 nm.

[d] Enantiomeric excess of (*R*)-**7** determined by HPLC analysis (Daicel Chiralcel OJ-H column) 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at 261 nm.

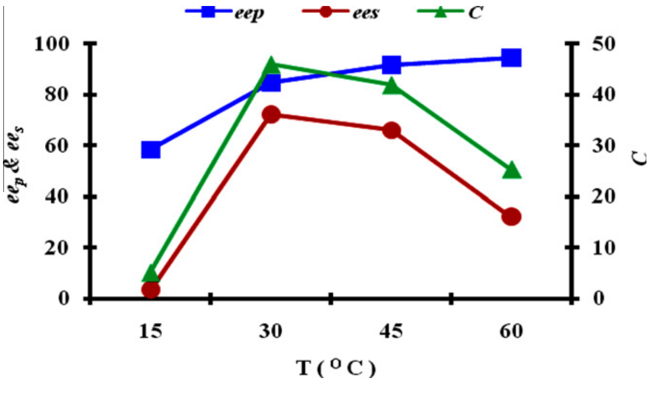


Figure 2. The effect of temperature on the CRL catalyzed transesterification of (*RS*)-**6** with vinyl acetate in toluene.

resolution was carried out at 15, 30, 45, and 60 °C and the conversion and the enantiomeric excess were determined using chiral HPLC after 9 h of reaction (Fig. 3). It was found that the conversion increased from 5.1% at 15 °C to 45.9% at 30 °C and then decreased to 25.3% at 60 °C. The ee of the substrate followed the same pattern, however the enantiomeric excess (ee) of the product had increased from 58.5% at 15 °C to 94.3% at 60 °C (Fig. 2).

2.3.6. Effect of enzyme concentration

It is known [20] that the enzyme concentration has a significant effect on the rate of conversion and ee. The reaction of (*RS*)-**6** with vinyl acetate was carried out using different concentrations of CRL enzyme (25, 50, 75, and 150 mg/mL) in toluene. Samples were collected from the reaction mixture at 9 h and the enzyme was removed by centrifugation. The percentage of conversion and enantiomeric excess were determined using chiral HPLC as described in Section 4.1.1. It was observed that when the enzyme concentration was increased, the conversion increased up to a certain level after which there was not any significant change in the conversion rate or ee. The maximum conversion (47.5%) was obtained at 50 mg/mL enzyme concentration with the highest ee_s values (Fig. 3).

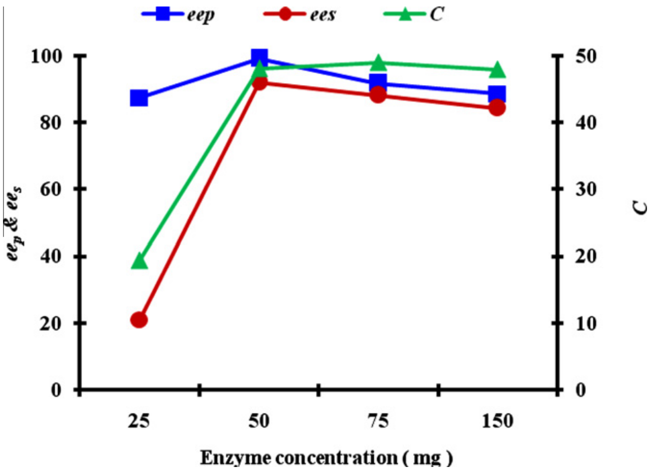


Figure 3. Effect of enzyme concentration on the CRL catalyzed transesterification of (*RS*)-**6** with vinyl acetate in toluene.

2.3.7. Effect of substrate concentration

It was necessary to study the effect of the substrate concentration on the activity and ee of the CRL for the resolution of (*RS*)-**6**. Various concentrations of the substrate were used, that is, 10, 20, 60, 120, and 180 mM. Samples were collected from the reaction mixture after 9 h and the enzyme was removed by centrifugation. The percentage conversion and enantiomeric excess were determined using chiral HPLC as described before. It was found that the maximum conversion was obtained with a substrate concentration of 10 mM (conversion 49.3%) and enantiomeric excess of

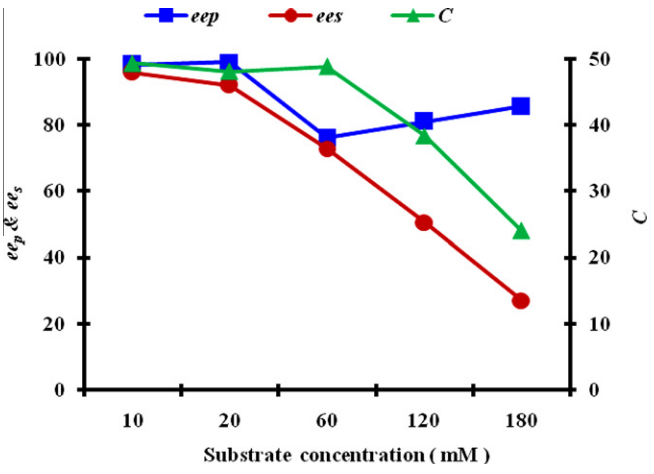
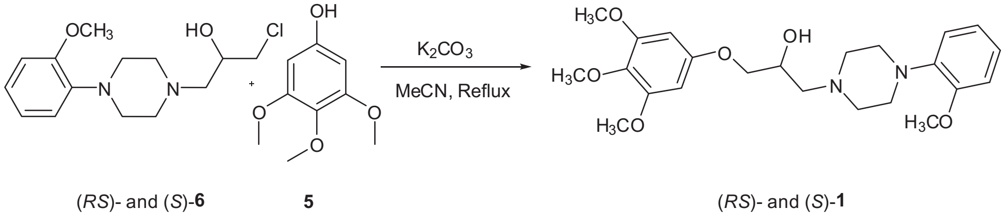


Figure 4. Effect of substrate concentration on the CRL catalyzed transesterification of (*RS*)-**6** with vinyl acetate in toluene.



Scheme 5. Synthesis of (*RS*)- and (*S*)-**1**.

ee_P = 98.3% and ee_S = 96% (Fig. 4). Therefore, a substrate concentration of 10 mM was used for further studies.

2.4. Synthesis of (*RS*)- and (*S*)-eciprazine 1

The (*RS*)- and (*S*)-enciprazine **1** drugs were synthesized by treatment of (*RS*)- and (*S*)-**6** with **5** using MeCN as the solvent and K_2CO_3 as the base. The reaction was carried out under reflux condition for 6 h at room temperature. The progress of the reaction was monitored by TLC. After the complete consumption of (*RS*)- and (*S*)-**6**, the product was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate and evaporated under vacuum and purified by flash chromatography to yield (*RS*)- and (*S*)-**1** (Scheme 5).

3. Conclusion

From our results, we can conclude that enantio-enriched (*S*)-enciprazine can be synthesized by a chemo-enzymatic route. The kinetic resolution of racemic alcohol (*RS*)-**6** to (*S*)-**6** by *Candida rugosa* lipase is a good example of the enantiomeric synthesis of an enantiomerically pure compound. Lipases from various sources were screened and the reaction conditions for the enantioselective resolution of (*RS*)-**6** in organic solvents were optimized. On a preparative scale, (*RS*)-**6** was transesterified quantitatively and both enantiomers were separated; (*S*)-**6** was used for the synthesis of (*S*)-**1** enciprazine. A new chemical method has also been developed for the synthesis of (*RS*)-enciprazine.

4. Experimental

4.1. General experimental

4.1.1. Analysis

Enzymatic reactions were carried out on a 'stackable Kuhner-shaker' at 200 rpm. ^{1}H NMR and ^{13}C NMR spectra were obtained with Bruker DPX 400 (^{1}H 400 MHz and ^{13}C 100 MHz), chemical shifts are expressed in δ units relative to the tetramethylsilane (TMS) signal as an internal reference in $CDCl_3$. IR spectra (wave number in cm^{-1}) were recorded on Nicolet FT-IR impact 400 instruments as either neat for liquid or KBr pellets for solid samples. Analytical TLC of all reactions was performed on Merck prepared plates. Column chromatography was performed using SRL silica gel (60–120 mesh). LC–MS analysis was carried out on Finninganmat LCQ instrument (USA) using a C-18 hypersil ODS (4.6 × 15 mm, 5 m) column. Optical rotations were measured in a Rudolph, Autopol^R IV polarimeter. The enantiomeric excesses (ee) were determined by HPLC performed on Shimadzu LC-10AT 'pump, SPD-10A UV–vis detector using a Chiralcel OJ-H column (0.46 × 250 mm; 5 μm, Daicel Chemical Industries, Japan) under the following conditions: mobile phase, 95:5:0.05; hexane/2-propanol/triethyl amine, 0.8 mL/min flow rate at column temperature, 25 °C at 262 nm.

4.1.2. Reagents

(*RS*)-Epichlorohydrin, (*S*)-epichlorohydrin, (*R*)-epichlorohydrin, 1-(2-methoxyphenyl) piperazine, 3,4,5-trimethoxy phenol, *Candida antarctica*, *Candida rugosa* L8525, *Candida cylindracea*, *Aspergillus niger,* and porcine pancreas lipase and chemicals were purchased from SIGMA (St. Louis, Missouri, USA). Solvents required for the synthesis and extraction were acquired from commercial sources and were either of analytical or commercial grade. HPLC grade solvents used for HPLC analysis were obtained from J.T. Baker, Rankem and Merck Ltd. Immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia*, immobilized lipozyme from *Mucor miehei*, lipase A, *Candida antarctica* lipase were purchased from Fluka™ and lipase AY 'Amano' 30 was purchased from Amano Chem Ltd. These were used without any further treatment.

4.2. Synthesis of (*RS*)-, (*R*)- *and* (*S*)-1-chloro-3-(4-(2-methoxy phenyl) piperazin-1-yl) propan-2-ol 6

Compound (*RS*)-**6** was synthesized by adding (*RS*)-epichlorohydrin **4** (0.93 g, 0.01 mol), 1-(2-methoxyphenyl) piperazine **3** (1.92 g, 0.01 mol) and LiBr (0.86 g, 0.01 mol) in 10 ml of water. The reaction was then allowed to stir at room temperature. The progression of the reaction was monitored by TLC. After completion of the reaction, the product was extracted with ethyl acetate and washed with a brine solution. The organic layer was separated, dehydrated using anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (60–120 mesh), eluting with ethyl acetate:hexane (15:85) to afford (*RS*)-**6**, which was then subjected to chiral HPLC analysis using a Chiralcel OJ-H column and the two enantiomers were eluted after 25.42 and 28.28 min (95:5:0.05; hexane/2-propanol/triethyl amine), respectively and in a ratio of 50.25:49.74.

(*RS*)-**6**: White solid, yield 2.41 g (85%); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.00–7.02 (m, 1H), 6.91–6.94 (m, 2H), 6.86–6.88 (m, 1H), 3.98–4.00 (m,1H), 3.87 (s, 3H), 3.56–3.65 (m, 2H), 3.11 (s, 4H), 2.86–2.88 (m, 2H), 2.66 (s, 2H), 2.56 (d, *J* = 5.6 Hz, 2H), 1.68 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 152.2, 140.9, 123.1, 120.9, 118.2, 111.1, 66.4, 61.0, 55.3, 53.5, 50.6, 47.0; FTIR (neat cm^{-1}) −3400 cm^{-1} (OH); LC–MS (*m*/*z*): 285.24, identical with an authentic sample.[21]

(*R*)-**6**: A white solid, yield 0.238 g (84%), 91% ee. $[\alpha]_D^{20} = -28.6$ (*c* 1.0, EtOH). The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_S = 25.42 min and t_R = 28.28 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 4.5% and 95.5%, respectively (91% ee).

(*S*)-**6**: White solid, yield 0.235 g (83%), 92% ee. $[\alpha]_D^{20} = +28.9$ (*c* 1.0, EtOH). The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_S = 25.42 min and t_R = 28.28 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 96% and 4%, respectively (92% ee).

4.3. Synthesis of (*RS*)-, (*R*)- *and* (*S*)-1-chloro-3-(4-(2-methoxyphenyl)piperazin-1-yl) propan-2-yl acetate 7

Compound (*RS*)-**7** was synthesized chemically via treatment of the corresponding alcohol of (*RS*)-**6** (0.142 g, 0.0005 mol) and acetic anhydride (2 ml) in the presence of pyridine [0.395 mg, 0.05 mmol] as a catalyst. The reaction mixture was stirred at 4 °C until the complete consumption of (*RS*)-**6**, as monitored by TLC. After the consumption of (*RS*)-**6**, the reaction mixture was poured into ice water (25 ml), which was acidified with HCl (3.0 M) to pH 1–2 and then extracted three times with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, and evaporated to yield (*RS*)-**7**.

(*RS*)-**7**: Yellow semisolid, yield 0.147 g (90%); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm): 6.97–6.99 (m, 1H), 6.89–6.92 (m, 2H), 6.85–6.86 (m, 1H), 3.86 (m 3H), 3.79 (s, 1H), 3.73 (s, 1H) 3.05 (s, 4H), 2.73 (s, 4H),2.66 (s, 2H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm): 170.3, 141.1, 122.9, 120.9, 118.2, 111.1, 70.3, 58.4, 55.3, 53.9, 50.6, 44.7, 21.0; FTIR (neat cm^{-1}): 1741 cm $^{-1}$ (C=O); LC–MS (*m*/*z*): 327.18. Next, (*RS*)-**7** was subjected to chiral HPLC analysis using a Chiralcel OJ-H column and the two enantiomers were eluted after 17.95 min and 23.57 min (95:5:0.05; hexane/2-propanol/triethyl amine), respectively and were present in a ratio of 49.89:50.11.

(*R*)-**7**: Yellow semisolid, yield 0.15 g (92%); $[\alpha]_D^{20} = -32.8$ (*c* 1.0, EtOH). The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_R = 23.57 min and t_S = 17.95 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 93.5% and 6.5%, respectively (87% ee).

(*S*)-**7**: Yellow semisolid, yield 0.148 g (91%); $[\alpha]_D^{20} = +33.5$ (*c* 1.0, EtOH). The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_R = 23.57 min and t_S = 17.95 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 5.5% and 94.5%, respectively (89% ee).

4.4. Enantioselective transesterification of (*RS*)-6

In a 10 mL round bottom flask containing magnetic beads, a mixture of (*RS*)-**6** (0.022 g, 0.08 mmol) in 4 mL of toluene and vinyl acetate (0.464 g, 5.40 mmol) was placed. Lipases from different sources (commercial lipase from lipase A, *Candida antarctica*, *Candida rugosa* L8525, *Candida cylindracea*, *Aspergillus niger*, porcine pancreas, and AY 'Amano' 30) were used to carry out the reaction. The round bottom flask was capped and placed on a magnetic stirrer which was maintained at room temperature. Immobilized lipase in sol-gel-Ak from *Pseudomonas cepacia*, immobilized lipozyme from *Mucor miehei*, and lipase acrylic resin from *Candida antarctica*, were individually taken into separate 10 mL conical flasks. The flasks were then capped and placed in a shaker, which was maintained at 30 °C (200 rpm). Samples (400 μL) were withdrawn from reaction mixture and the conversion and enantiomeric excess of the reaction were monitored by HPLC.

4.5. Optimization of the transesterification reaction

The effect of different organic solvents such as diisopropyl ether, diethyl ether, dichloromethane, benzene, heptane, *iso*-octane, and toluene on the transesterification of (*RS*)-**6** was studied. The optimum temperature was determined by carrying out the reaction at various temperatures in the range of 15–60 °C. In order to determine the effect of different acyl donors, various acyl donors such as ethyl acetate, acetic anhydride, and vinyl acetate (5.40 mmol) were used. In order to optimize the enzyme concentration with respect to a constant amount of substrate, various enzyme concentrations (25, 50, 75, and 150 mg/mL) were used. Finally, in order to optimize the substrate concentration with respect to constant enzyme concentration (50 mg/ml), various substrate concentrations (10 20, 60, 120, and180 mM) were used. The samples were taken at regular time intervals and analyzed for the enantioselectivity of the transesterification reaction.

4.6. Preparative-scale transesterification reaction

The resolution of (*RS*)-**6** was carried out on a preparative scale under optimized condition. The reaction was performed by subjecting 45 mL (10 mM substrate) of the reaction mixture to resolution by CRL at 30 °C using vinyl acetate as the acyl donor in toluene. After 9 h (49.3% conversion), the reaction mixture was filtered off and the enzyme preparation was washed with a solvent. The solvent was evaporated under reduced pressure and the resulting dried residue was subjected to flash chromatography using ethyl acetate: hexane (15:85 v/v) as the mobile phase. We observed that after 9 h the isolated yield of (*S*)-**6** was 41.8% with enantiomeric excess ee$_S$ = 96%, (Chiralcel OJ-H) and that of (*R*)-**7** was 44.7% with enantiomeric excess ee$_P$ = 98.3% (Chiralcel OJ-H).

(*S*)-**6**: White solid, yield 0.105 g (41.8%), 96% ee. $[\alpha]_D^{20} = +30.15$ (*c* 1.0, EtOH). The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_S = 25.42 min and t_R = 28.28 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 98% and 2%, respectively (96% ee).

(*R*)-**7**: Yellow colored semisolid, yield 0.115 g (44.7%); $[\alpha]_D^{20} = -36.2$ (*c* 1.0, EtOH). The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_R = 23.57 min and t_S = 17.95 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 99.15% and 0.85%, respectively (98.3% ee).

4.7. Synthesis of (*RS*)- and (*S*)-enciprazine 1

The (*RS*)- and (*S*)-enciprazine **1** was synthesized by reaction of (*RS*)- and (*S*)-**6** (0.0569 g, 0.0002 mol) and trimethoxyphenol **5** (0.0272 g, 0.0002 mol) using MeCN as the solvent and K$_2$CO$_3$ (0.0552 g, 0.0004 mol) as the catalyst. The reaction was carried out under reflux conditions for 6 h. Progress of the reaction was monitored by TLC. After the complete consumption of (*RS*)- and (*S*)-**6**, the mixture was diluted with ethyl acetate (15 mL) and washed with water. The organic layer was separated, dried over anhydrous Na$_2$SO$_4$, and concentrated under vacuum. The residue was purified by passing through a column of silica (60–12 mesh) and eluting with ethyl acetate/hexane (15:85) to obtain (*RS*)- and (*S*)-enciprazine **1**.

(*RS*)-Enciprazine **1**: Yellow viscous liquid yield 0.082 g (95%); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm): 6.99–7.04 (m, 1H), 6.93–6.96 (m, 2H) 6.86–6.91 (m, 1H), 6.20 (s, 2H), 4.12–4.15 (m, 1H), 3.98–3.99 (d, *J* = 4.8 Hz, 2H), 3.84–3.87 (m, 9H), 3.79 (s, 3H), 3.11 (s, 4H), 2.89–2.92 (m, 2H), 2.62–2.69 (m, 4H); ^{13}C NMR (100 MHz, CDCl3): δ (ppm): 155.4, 153.6, 152.2, 141.2, 132.4, 123.0, 122.9, 121.0, 118.2, 111.1, 92.3, 70.6, 65.4, 61.0, 60.5, 56.0, 55.3, 53.5, 50.7; FTIR (neat cm^{-1}): 3394 cm^{-1}(OH); LC–MS (*m*/*z*): 433.37. identical with an authentic sample.[10] Next, (*RS*)-**1** was subjected to chiral HPLC analysis using a Chiralcel OJ-H column and the two enantiomers were eluted after 29.43 min and 30.98 min (95:5:0.05; hexane/2-propanol/triethyl amine), respectively and were present in a ratio of 49.9:50.1.

(*S*)-**1**: Yield .0812 g (94%).$[\alpha]_D^{20} = +29$ (*c* 0.5, EtOH) {lit[22] $[\alpha]_D^{20} = +3.0$ (*c* 0.5, EtOH)}. The product was then subjected to chiral HPLC analysis using chiral OJ-H column, the two enantiomers were eluted at t_R = 30.98 min and t_S = 29.43 min (95:5:0.05; hexane/2-propanol/triethyl amine) with peak areas of 2.5% and 97.5%, respectively (95% ee).

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